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(21) International Application Number: PCT/US93/09094 (22) International Filing Date: 24 September 1993 (24.09.93) (30) Priority data: 951,752 28 September 1992 (28.09.92) US (71) Applicant (for all designated States except US): MIDWEST RESEARCH INSTITUTE [US/US]; 425 Volker Boulevard, Kansas City, MO 64110 (US). (72) Inventors; and (75) Inventors/Applicants (for US only) : GROHMANN, Karel [US/US]; 1455 Howard Lake Drive, N.W., Winter Haven, FL 33881 (US). WYMAN, Charles, E. [US/US]; 2225 South Queen, Lakewood, CO 80227 (US). HINMAN, Norman, D. [US/US]; 7674 South Race Street, Littleton, CO 80122 (US).		(74) Agent: O'CONNOR, Edna, M.; Senior Patent Counsel, National Renewable Energy Laboratory, 1617 Cole Boulevard, Golden, CO 80401 (US). (81) Designated States: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>With amended claims.</i>
(54) Title: FERMENTATION OF CELLULOSE AND HEMICELLULOSE IN CORN FIBER AND DISTILLERS DRIED GRAINS WITH SOLUBLES TO ETHANOL (57) Abstract A method of converting carbohydrates in corn fiber and distillers dried grain and solubles to increase the yield of ethanol, comprising pretreating a feedstock of corn fiber or distillers dried grain and solubles with dilute sulfuric acid or other means to convert hemicellulose into soluble arabinose, xylose, and other sugars under conditions that preserve protein content; treating the pretreated material with cellulase and possibly hemicellulase enzymes to convert cellulose and possibly hemicellulose portions into fermentable sugars; and treating the enzyme treated materials with fermenting microbes to ferment the sugars produced through pretreatment and enzymatic action to alcohol.		

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DescriptionFermentation Of Cellulose And Hemicellulose In Corn FiberAnd Distillers Dried Grains With Solubles To Ethanol

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The United States Government has rights in this invention pursuant to Contract No. DE-AC02-83CH10093 between the United States Department of Energy and National Renewable Energy Laboratory, Division the Midwest Research Institute.

Technical Field

The invention relates to a method for converting the hemicellulose and cellulose content of corn fiber and distillers dried grains and solubles (DDGS) into ethanol.

Background Art

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There are three major processes for production of products from corn kernels: (1) wet milling-based processes, (2) whole grain process, and (3) dry milling process.

The wet milling process consists of the following:

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First, corn is steeped with water, SO₂, and lactic acid to break the bonds between the starch and protein in the endosperm. Following steeping, the corn is put through a series of milling and particle separation steps to produce: germs (high in oil content), fiber (high in cellulose and hemicellulose), gluten (60% protein), highly purified starch (>99% pure), and corn steep liquor (high in sugar and minerals). The germs are sent to a corn oil plant where crude corn oil and germ meal are produced. The corn oil is refined and the germ meal is typically blended with the fiber and corn steep liquor to be

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sold as corn gluten feed, an animal feed that contains about 21% protein. The gluten is sold as animal feed called corn gluten meal. Starch, the primary product from the wet milling process, can be used to produce a variety of products. To produce starch products the starch is sent to a starch plant where it is modified chemically and then dried. To produce maltodextrins and corn syrups, the starch is treated with acid, acid plus enzyme, or enzymes only to partially hydrolyze the starch to lower molecular weight polymers of glucose. To produce dextrose (glucose), the starch is totally hydrolyzed to dextrose with enzymes (alpha-amylase followed by glucomylase). Various qualities of dextrose are sold: crystalline, high dextrose syrups, etc. High fructose corn syrup is produced from dextrose by enzymatic isomerization. To produce ethanol, dextrose syrup is fed to a fermenter where ethanol is produced. The dilute ethanol stream from the fermenter is distilled to recover high purity ethanol.

Typical yields of such products for wet milling per bushel of corn are shown below.

15			Starch 31.4 lb (67%)
	Wet Milling		Oil 1.9 lb (4 %)
	→ Process →		Germ Meal 1.9 lb (4%)
1 Bushel Corn			Fiber 5.4 lb (11.5%)
(47.32 lb dry wt)			Gluten 2.6 lb (5.5%)
20			Steepwater 3.8 lb (8%)

In the whole grain process, which is intended primarily for ethanol production (industrial, beverage and fuel), corn is first milled to open the grain in preparation for the "mashing" or cooking process. Starch in the mash is liquified and saccharified with

enzymes, and fermented to ethanol and CO₂ by the action of yeast. The resulting "beer" is then distilled to recover hydrous ethanol, and further processed to produce anhydrous ethanol. In addition to ethanol, a whole grain plant yields as a coproduct distillers dried grains with solubles (DDGS), commonly used as a protein animal feed, as a result of drying stillage from beer distillation. The three products (ethanol, CO₂ and DDGS) are produced in approximately equal weight per bushel, with each accounting for approximately one-third of the initial weight of the corn. Using current technology, ethanol producers can produce about 2.50-2.65 gallons of undenatured fuel-grade ethanol plus 16.5-17.5 pounds of DDGS from one bushel of corn. Carbon dioxide (CO₂) may also be collected from fermentation tanks for use in such applications as food processing, dry ice production, and tertiary recovery of oil.

In most dry milling processes, the corn is tempered by soaking it in water for a short time and the product is then processed in a degerminator, in which the hull and germ are stripped from the endosperm. The hulls and germs are then passed through a process where the hulls are removed, so that the germ can be expelled or hexane-extracted to remove the oil, and the spent germ cake becomes a coproduct which is mixed with the hulls and other fractions to become "hominy feed." The main portion of the endosperm is processed to produce prime grits, meals and flours.

U.S. patent 4,752,579 discloses a method of making D-glucose, D-xylose, and L-arabinose, in combination or either one alone from a readily abundant material without the need for any delignifying pretreatment comprising, hydrolyzing corn kernel hulls with acid at elevated temperature with subsequent enzymatic hydrolysis of the hydrolysate. In particular, acid hydrolysis is conducted at a temperature from about 85° to about 110°C.

A process of making ethanol from a cellulosic material using plural ferments is described in U.S. patent 4,009,075. The process comprises steam sterilization of the

cellulosic material, which subjects the sterilized cellulosic material to digestion and fermentation reaction with an inoculum mixture comprising cellulase enzyme and a yeast. The cellulosic material is then converted to simple sugars with conversion of said sugars to ethanol, recovering ethanol from said digestion and fermentation reaction by vacuum stripping, and recycling the inoculum enzyme-containing residual liquid for reuse to digest subsequent charges of cellulosic material, said digestion of the cellulosic material to simple sugars and the fermentation reaction of the sugars to ethanol being carried out concurrently.

Ethyl alcohol is manufactured from Indian corn in U.S. patent 2,132,250 by first leaching Indian corn with hot water containing sulfur dioxide to dissolve water soluble substances and amino acids, then washing the thus-leached corn with water to free it from water-soluble substances (including amino acids). The wash waters are then subjected to the Abderhalden "ninhydrine" test to determine the presence of amino acids, and the washing continues with fresh water until the test gives negative results to produce leached corn freed from amino acids. The leached corn is cracked to produce a mass containing cracked corn and oil-bearing germs, and said germs are separated from said cracked corn. The degerminated cracked corn freed from amino acids and containing hulls is subject to the action of heat in the presence of water and a small amount of malt to liquefy and then to saccharify the same, cooling the liquefied and saccharified mass to about 80° F. The said cooled mass is fermented at about 65° F in the presence of "distiller's seed yeast" to produce ethyl alcohol, and distilling the fermented mass to obtain ethyl alcohol devoid of fusel oil.

U.S. patent 4,287,304 pertains to a process for converting starch derived from dry milled whole corn to fermentable sugar to provide substrate for the thermally efficient large scale production of ethanol. An aqueous slurry of the starch is subjected to a mild

hydrolysis to provide a sterile partial starch hydrolysate containing the water insoluble protein and oil and the water soluble components of the starch in an unaltered condition. The slurry is then separated into an aqueous partial starch hydrolysate portion containing a part of the water soluble components, and a water insoluble protein and oil portion
5 containing the remaining part of the water soluble components. The aqueous partial starch hydrolysate portion is subjected to further hydrolysis and the resulting aqueous solution of fermentable sugar, together with part of the water soluble component of the original starch feed, is conveyed to a fermentation unit where conversion of the sugar to ethanol and further hydrolysis of any remaining partial starch hydrolysate to fermentable sugar takes
10 place.

However, the current state of the art in the ethanol industry converts only the starch portion of corn into ethanol and this limits the ethanol yield per bushel of corn. The cellulose and hemicellulose content of a corn kernel is left unconverted and constitutes part of the animal feed coproducts.

15 Disclosure of the Invention

It is an object of the invention to provide novel means for converting more than the starch portion of corn into ethanol in order to increase the ethanol yield per bushel of corn.

In general, in the non-wet grain process plant, a feedstock of corn milling
20 byproducts containing cellulose and hemicellulose is first pretreated with an acid such as dilute H_2SO_4 or other means to convert the hemicellulose portion into soluble xylose, arabinose, and other sugars. This is done under reaction conditions which will preserve the protein content of the feedstock where protein content is significant (such as with a DDGS feedstock). The pretreated feedstock is then treated with a cellulase to convert the

cellulose into fermentable sugars, and the sugars from pretreatment and cellulose ethers are then fermented into ethanol by the use of a suitable combination of bacteria, yeast or fungi. Alternatively, the xylose can be converted to xylulose by xylose isomerase enzyme fermented to ethanol by an appropriate microorganism.

5 In the case of a wet grain process plant where there is a fermenter that is primarily used to ferment saccharified corn starches into ethanol, the fermenter contents may be economically supplemented with the cellulose and hemicellulose-derived sugars for conversion to ethanol.

10 In other cases, the pretreated feedstock (via H_2SO_4 or other means) is subjected to a simultaneous saccharification and fermentation (SSF) process in the presence of cellulase and enzymes, as well as xylose, arabinose, glucose, and other sugar fermenting microbes to simultaneously break down the cellulose into glucose and ferment the sugars into ethanol. Alternatively, isomerase enzyme may be used to convert xylose into xylulose which yeast can ferment into ethanol.

15 **Brief Description of the Drawings**

Figure 1 depicts the main components of a typical corn kernel.

Figure 2 depicts a schematic of the whole grain process.

Figure 3 depicts a schematic of the wet milling process.

Detailed Description of the Invention

20 One of the major processes for the production of products from corn kernels is the whole grain process, and a major product from this process is DDGS, an animal feed containing about 27% protein. The price of DDGS is largely set by its protein content thereby giving less value to the appreciable cellulose and hemicellulose content.

Another process that utilizes corn kernels is the wet milling process, which also produces an animal feed (corn gluten) from its corn fiber and germ meal byproduct streams. Like DDGS, the price of this animal feed is dependent on its protein content, thereby giving less value to the cellulose, hemicellulose and other carbohydrate materials.

5 Because the ethanol industry currently converts only the starch portion of corn into ethanol, the present invention method of additionally converting hemicellulose and cellulose into ethanol increases the percentage of ethanol yields.

In commencing the process of the invention, a quantity of fiber and/or germ meal streams is obtained from a corn wet milling process, and the composition of
10 cellulose, hemicellulose, starch and protein is measured.

The germ meal, which is present in amounts of about 1.9 lbs/bushel of corn, had the following measurements:

	Hemicellulose 32%	}	
		}	
15	Cellulose 13%	}	
		}	
	Starch 20%	}	65% carbohydrate
		}	(1.2 lbs)
	Protein 26%	}	
20		}	
	Other 9%	}	

The fiber, which is present in amounts of about 5.4 lbs/bushel of corn had the following measurements:

	Hemicellulose 30%	}	
25		}	
	Cellulose 10%	}	
		}	
	Starch 22.5%	}	62.5% carbohydrate
		}	(3.4 lbs)
30	Protein 12.5%	}	
		}	
	Other 25%	}	

5 Corn steep liquor, which is present in amounts of about 3.8 lbs/bushel of corn
had the following measurements:

Protein 47%

Lactic Acid 23%

5 Sugars 6%

Other 24%

10 The combined streams of germ meal and fiber contain 2.23 lbs of
hemicellulose, 0.79 lbs of cellulose, and 1.59 lbs of starch per bushel of corn, and the
objective is to substantially convert a substantial fraction of the total carbohydrate into
ethanol.

 Corn gluten feed, which may be one of the starting feedstocks in the process of
the present invention is composed of the germ meal, fiber and steep liquor.

15 Another feedstock in the process of the present invention is DDGS, which is a
product derived from the whole grain process. The composition of DDGS is typically as
follows:

Component	% Dry Basis
Protein	29.7
Fat	8.8
20 Cellulose (Acid Det.)	18.7
Hemicellulose	25.3*
Ash	4.9
Fiber (Neut. Det.)	44.0

25 *Estimated by subtracting Acid Det.
from Neut. Det.

 In general, in the invention process, these carbohydrates are subjected to
pretreatment by acid or other means, washed, and saccharified by cellulose enzyme either

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The yield of ethanol in Example 2 was 14% more than the yield of ethanol in Example 3.

When all of the hemicellulose and cellulose in DDGS is also converted into ethanol, an additional 0.37 gallons of ethanol for every bushel of corn processed is obtained compared to standard yields.

Comparable results were obtained when the hemicellulose and cellulose of corn fiber were also converted into ethanol in Example 1.

Claims

1. A method of converting carbohydrates in corn fiber to increase the yield of ethanol, comprising:

5 a) pretreating a corn fiber feedstock with dilute sulfuric acid or other suitable means to convert the hemicellulose portions of said fiber into soluble arabinose, xylose, or other sugars under conditions that preserve the protein content of said fiber;

b) treating the pretreated fiber of step a) with cellulase enzymes to convert the cellulose portions into fermentable sugars; and

10 c) treating material of step b) with fermenting microbes to ferment the sugars from cellulose and hemicellulose in said fiber to ethanol.

2. The process of claim 1, wherein said conditions comprise pretreating with dilute sulfuric acid at a concentration of up to about 0.5% and a temperature range of from about 140° C to about 200° C.

15 3. The process of claim 2, wherein in step b) there is added to a vessel containing loadings of cellulase quantities of substrate of material from step a), and in step c) a mixed culture of an ethanol tolerant yeast such as S. cerevisiae and a cellobiose fermenting yeast B. clausenii.

20 4. The process of claim 3, wherein in step b) there is added to a vessel containing loadings of cellulase and substrate of material from step a) a fermenting organism such as S. cerevisiae to simultaneously ferment sugar to ethanol.

5. The process of claim 3, wherein an amount of cellulose fermenting yeast such as Brettanomyces clausenii or B. custersii is added to the material from steps a) and b) in sufficient amounts to simultaneously convert about 5 to about 10% cellulose at a temperature of about 37°C-40°C.

5 6. The process of claim 5, wherein said corn fiber comprises on a dry basis:

22.5% - starch

12.5% - protein

3.0% - fat

10.0% - cellulose

10 30.0% - hemicellulose

0.5% - lignin

2.0% - ash

45.0% = fiber

15 7. The process of claim 2, wherein in step b) there is added to a vessel containing loadings of cellulase, small quantities of substrate of material from step a), and in step c) a mixed culture of S. cerevisiae and cellobiose fermenting yeast B. custersii.

8. The process of claim 2, wherein in step b) there is added xylose isomerase enzyme to convert xylose to xylulose and a yeast such as Schizosaccharomyces pombe that ferments xylulose into ethanol.

9. The process of claim 2, wherein in step b) an organism is used such as recombinant Escherichia coli that can ferment all sugars to ethanol.

10. A method of converting carbohydrates in distillers dried grain with solubles (DDGS) to increase the yield of ethanol, comprising:

5 a) pretreating a distillers dried grain with solubles feedstock with dilute sulfuric acid or other means to convert the hemicellulose portions of said grain into soluble arabinose, xylose, and other sugars under conditions that preserve the protein content of said grain with solubles;

10 b) treating the pretreated grain and solubles of step a) with cellulase enzymes to convert cellulose portions into fermentable sugars; and

c) treating material of step b) with fermenting microbes to ferment the sugars from cellulose and hemicellulose in said grain with solubles to ethanol.

11. The process of claim 10, wherein said conditions comprise dilute sulfuric acid at a concentration of up to about 0.5% and a temperature range of from about 140 to
15 about 200° C.

12. The process of claim 11, wherein in step b) there is added to a vessel containing loadings of cellulase, small quantities of substrate of material from step a), and in step c) a mixed culture of S. cerevisiae and a cellobiose fermenting yeast such as B. clausenii.

13. The process of step 12, wherein B. clausenii, B. custersii or other cellobiose fermenting yeast is added in sufficient amounts to convert about 10 to about 19% cellulose at a temperature of about 37° C-40° C.

14. The process of claim 13, wherein said grain and said solubles comprise
5 on a dry basis:

29.7% - protein

8.8% - fat

18.7% - cellulose

25.3% - hemicellulose

10 4.9% - ash

44.0% = fiber

15. The process of claim 11, wherein in step b) there is added to a vessel containing loadings of cellulase, small quantities of substrate of material from step a), and in step c) a mixed culture of S. cerevisiae and cellobiose fermenting yeast B. custersii.

15 16. The process of claim 11, wherein in step b) there is added xylose isomerase enzyme to convert xylose to xylulose and a yeast such as S. pombe that ferments xylulose into ethanol.

17. The process of claim 11, wherein in step b) an organism is used such as recombinant E. coli that can ferment all sugars to ethanol.

AMENDED CLAIMS

[received by the International Bureau
on 10 January 1994 (10.01.94); original claims 1-17 replaced
by amended claims 1-17 (4 pages)]

1. A method of converting carbohydrates in corn fiber to increase the yield of ethanol, comprising:

a) pretreating a corn fiber feedstock with dilute sulfuric acid to convert the hemicellulose portions of said fiber into soluble arabinose, xylose, or other sugars that preserve the protein content of said fiber;

b) treating the pretreated fiber of step a) with cellulase enzymes to convert the cellulose portions into fermentable sugars;

c) treating material of step b) with fermenting microbes to ferment the sugars from cellulose and hemicellulose in said fiber to ethanol; and

d) recovering ethanol.

2. The process of claim 1, wherein said pretreating with dilute sulfuric acid is at a concentration of up to about 0.5% and a temperature range of from about 140 to about 200°C.

3. The process of claim 2, wherein in step b) there is added to a vessel containing cellulase quantities of substrate of material from step a), and in step c) a mixed culture of an ethanol tolerant yeast such as S. cerevisiae and a cellobiose fermenting yeast Brettanomyces clausenii.

4. The process of claim 3, wherein in step b) there is added to a vessel containing cellulase and substrate of material from step a) a fermenting organism of Saccharomyces cerevisiae to simultaneously ferment sugar to ethanol.

5. The process of claim 3, wherein an amount of cellulose fermenting yeast such as Brettanomyces clausenii or B. custersii is added to material from steps a) and b) in sufficient amounts to simultaneously convert about 5 to about 10% cellulose at a temperature of about 37°C-40°C.

6. The process of claim 5, wherein said corn fiber comprises on a dry basis:

- 22.5% - starch
- 12.5% - protein
- 3.0% - fat
- 10.0% - cellulose
- 30.0% - hemicellulose
- 0.5% - lignin
- 2.0% - ash
- 45.0% - fiber

7. The process of claim 2, wherein in step b) there is added to a vessel containing cellulase, quantities of substrate of material from step a), and in step c) a mixed culture of S. cerevisiae and cellobiose fermenting yeast B. custersii.

8. The process of claim 2, wherein in step b) there is added a xylose isomerase enzyme to convert xylose to xylulose and a yeast of Schizosaccharomyces pombe that ferments xylulose into ethanol.

9. The process of claim 2, wherein in step b) an organism of recombinant *Escherichia coli* that can ferment all sugars to ethanol is used.

10. A method of converting carbohydrates in distillers dried grain with solubles (DDGS) to increase the yield of ethanol, comprising:

a) pretreating a distillers dried grain with solubles feedstock with dilute sulfuric acid to convert the hemicellulose portions of said grain into soluble arabinose, xylose, and other sugars under conditions that preserve the protein content of said grain with solubles;

b) treating the pretreated grain and solubles of step a) with cellulase enzymes to convert cellulose portions into fermentable sugars;

c) treating material of step b) with fermenting microbes to ferment the sugars from cellulose and hemicellulose in said grain with solubles to ethanol; and

d) recovering ethanol.

11. The process of claim 10, wherein said pretreating with dilute sulfuric acid is at a concentration of up to about 0.5% and a temperature range of from about 140 to about 200°C.

12. The process of claim 11, wherein in step b) there is added to a vessel containing cellulase, small quantities of substrate of material from step a), and in step c) a mixed culture of S. cerevisiae and a cellobiose fermenting yeast such as Brettanomyces clausenii.

13. The process of claim 12, wherein Brettanomyces clausenii, B. custersii or other cellobiose fermenting yeast is added in sufficient amounts to convert about 10 to about 19% cellulose at a temperature of about 37°C-40°C.

14. The process of claim 13, wherein said distillers dried grain with solubles comprise on a dry basis:

29.7% - protein
8.8% - fat
18.7% - cellulose
25.3% - hemicellulose
4.9% - ash
44.0% = fiber

15. The process of claim 11, wherein in step b) there is added to a vessel containing cellulase, small quantities of substrate of material from step a), and in step c) a mixed culture of S. cerevisiae and cellobiose fermenting yeast B. custersii.

16. The process of claim 11, wherein in step b) there is added xylose isomerase enzyme to convert xylose to xylulose and a yeast of Schizosaccharomyces pombe that ferments xylulose into ethanol.

17. The process of claim 11, wherein in step b) an organism of recombinant *Escherichia coli* is used to ferment all sugars to ethanol.

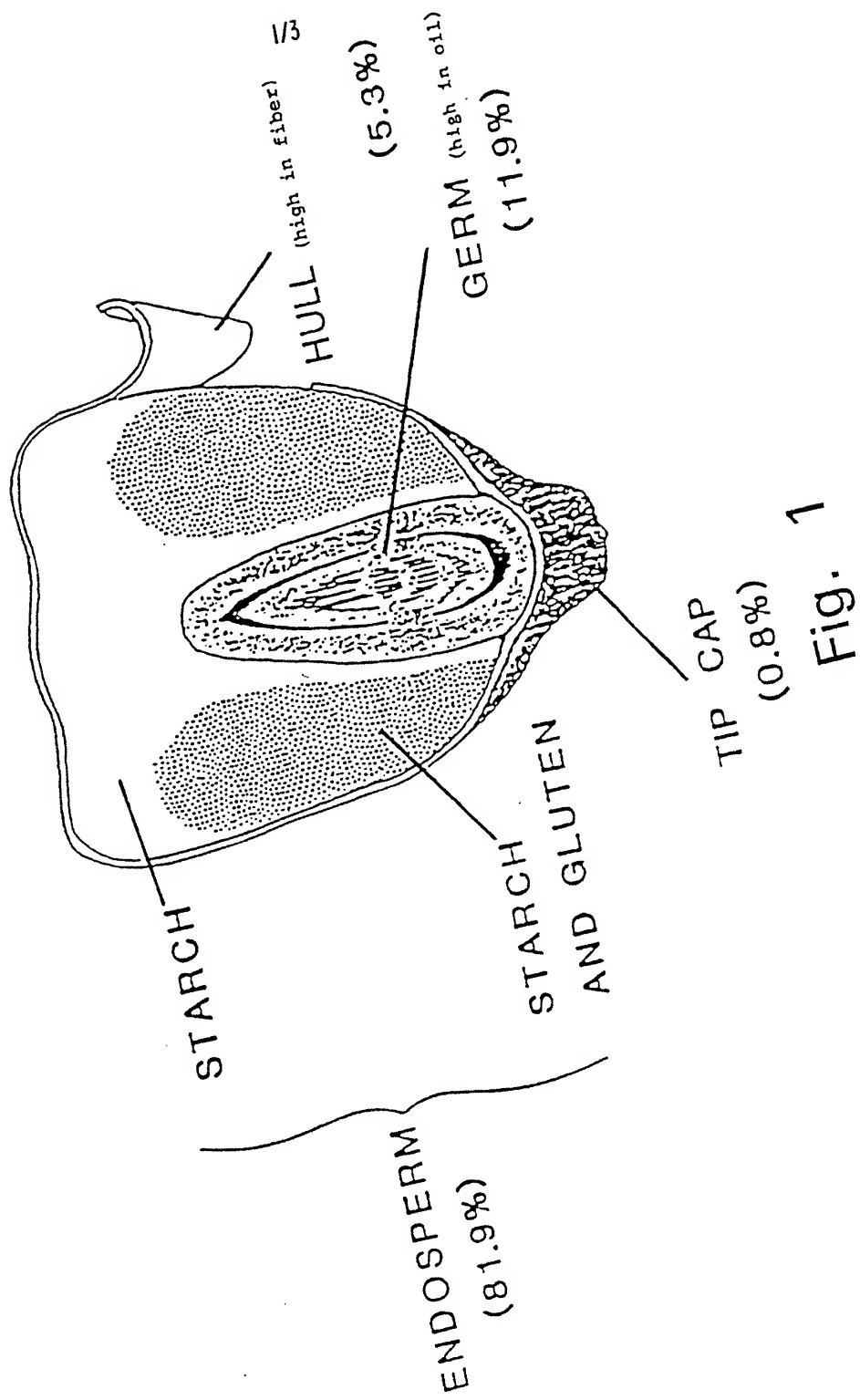


Fig. 1

SUBSTITUTE SHEET

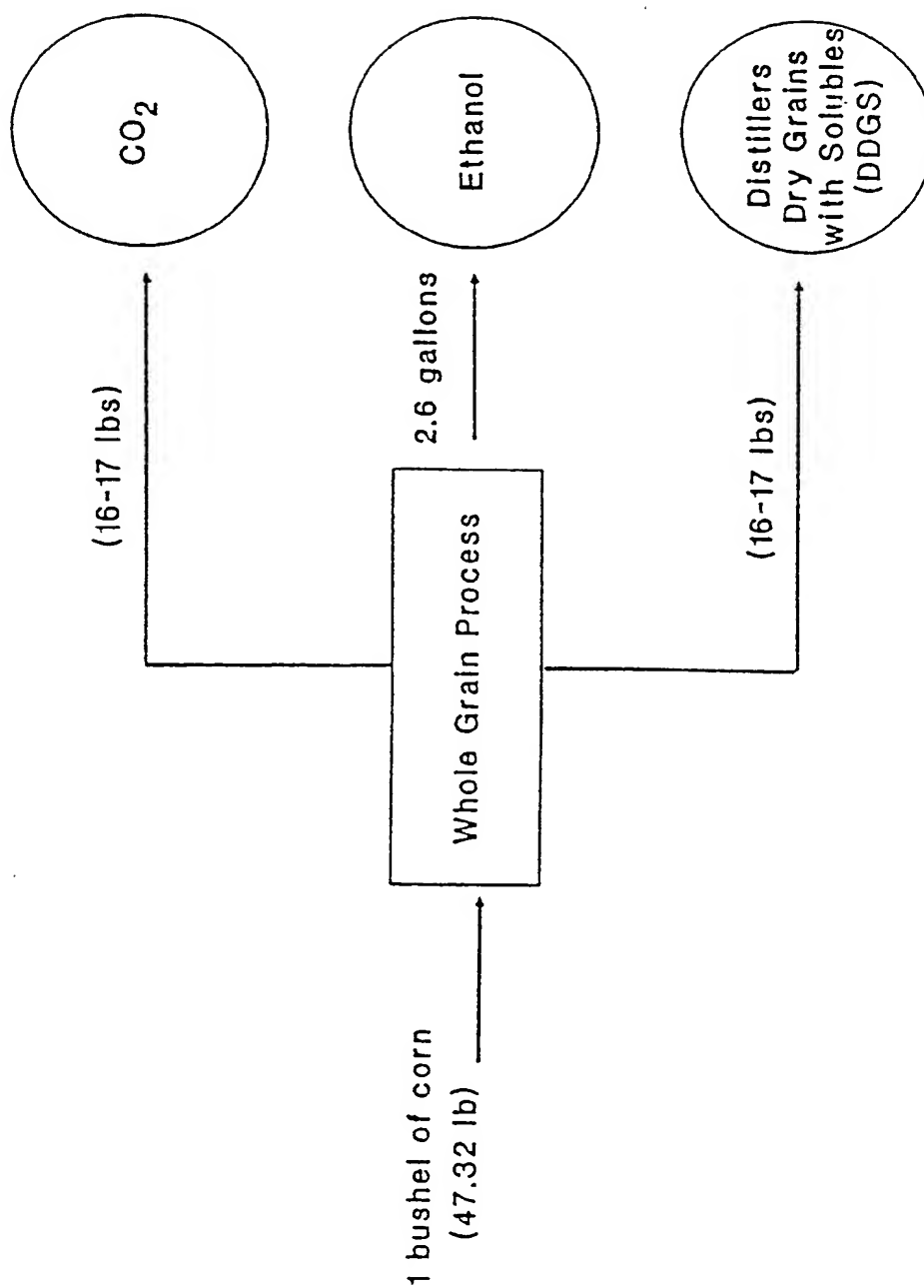


Fig. 2

SUBSTITUTE SHEET

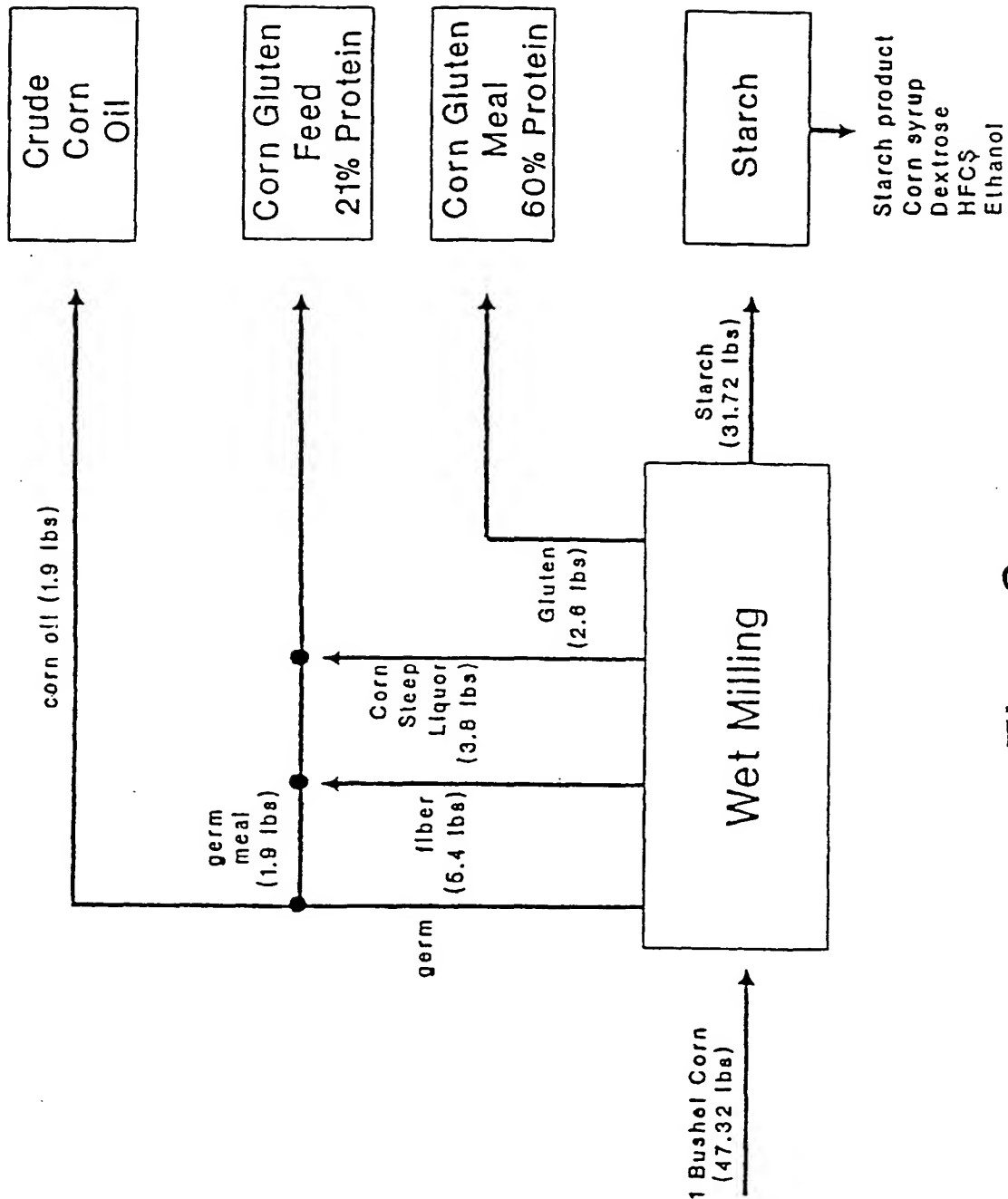


Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/09094

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12P 07/14, 07/08, 07/10

US CL :435/162, 163, 165; 426/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/162, 163, 165; 426/11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,378,432 (Castelli et al.) 29 March 1983, whole document.	1-17
Y	US, A, 4,752,579 (Arena et al.) 21 June 1988, whole document.	1-9
Y	US, A, 4,578,353 (Assarsson et al.) 25 March 1986, whole document.	1-17
Y	US, A, 5,000,000 (Ingram et al.) 19 March 1991, whole document.	9 and 17
Y	US, A, 5,100,791 (Spindler et al.) 31 March 1992, whole document.	3-7, 12-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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05 NOVEMBER 1993

Date of mailing of the international search report

02 DEC 1993

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INTERNATIONAL SEARCH REPORT

International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,460,687 (Ehnstrom) 17 July 1984, whole document.	10-17
Y	J. Ferment. Technol., Volume 64, No. 2, issued 1986, Koba, Y. et al., "Preparation of <u>Koji</u> from Corn Hulls for Alcoholic Fermentation without cooking, pages 175-178, whole document.	1-9
Y	Biotechnology, Vol. 3, issued 1983, Kosaric et al., "Ethanol Fermentation", pages 257-385, especially 322.	8 and 16

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